Appl. No. 10/700,297 Amdt. dated August 1, 2007 Response to Notice of Allowance of May 2, 2007

Amendments to the Specification:

Please replace the paragraph beginning at page 16, line 10, with the following:

--Figure 1 (upper profile) shows the mass spectrum of the three peptides (human histidine rich glycoprotein metal-binding domains (GHHPH)₂G (SEQ ID NO:1) (1206 Da), (GHHPH)₅G (SEQ ID NO:2) (2904 Da), and human estrogen receptor dimerization domain (D473-L525) (6168.4 Da)) desorbed in the presence of neutralized energy absorbing molecules (sinapinic acid, pH 6.2). Figure 1 (lower profile) shows the sequential *in situ* metal (Cu)-binding of the peptides in the presence of neutral energy absorbing molecules.--

Please replace the paragraph beginning at page 17, line 3, with the following:

--Figure 3 shows a composite mass spectra of the (GHHPH)₅G peptide (SEQ ID NO:2) (2904 Da) before (lower profile) and after (upper profile) *in situ* digestion by carboxypeptidase P in the presence of neutralized energy absorbing molecules (sinapinic acid, pH 6.2). (GHHPH)₅ = SEQ ID NO:3.--

Please replace the paragraph beginning at page 47, line 19, with the following:

--1. Sinapinic acid (Aldrich Chemical Co., Inc., Milwaukee, WI) is suspended in water at 20 mg/ml (pH 3.88) and neutralized with triethylamine (Pierce, Rockford, IL) to pH 6.2-6.5. An aqueous mixture (1 μl) of synthetic peptides, containing human histidine rich glycoprotein metal-binding domains (GHHPH)₂G (SEQ ID NO:1) (1206 Da), (GHHPH)₅G (SEQ ID NO:2) (2904 Da), and human estrogen receptor dimerization domain (D473-L525) (6168.4 Da) is mixed with 2 μl sinapinic acid (20 mg/ml water, pH 6.2) on a probe tip and analyzed by laser desorption time-of-flight mass spectrometry. After acquiring five spectra (average 100 laser

Appl. No. 10/700,297 Amdt. dated August 1, 2007 Response to Notice of Allowance of May 2, 2007

shots per spectrum), the probe is retrieved, 2 μl of 20 mm Cu(SO)₄ is added and the sample is reanalyzed by mass spectrometry. Figure 1 (upper profile) shows the mass spectrum of the three peptides desorbed in the presence of neutralized energy absorbing molecules. Figure 1 (lower profile) shows the *in situ* metal-binding of the peptides in the presence of neutral energy absorbing molecules. The (GHHPH)₂G (SEQ ID NO:1) peptide can bind at least 4 Cu(II)[[]], the (GHHPH)₅G (SEQ ID NO:2) peptide can bind at least 5 Cu(II) and the dimerization domain can bind at least 1 Cu(II) under the present experimental conditions. Similar result is obtained with α-cyano-4-hydroxycinnamic acid (20 mg/ml water) neutralized to pH 6.5.--

Please replace the paragraph beginning at page 49, line 13, with the following:

--3. An aliquot of 1 μl of (GHHPH)₅G (SEQ ID NO:2) peptide (2904 Da) is mixed with 2 μl of sinapinic acid (20 mg/ml water) neutralized to pH 6.2, and analyzed by laser desorption time-of-flight mass spectrometry. After acquiring five spectra (average 100 laser shotsper shots per spectrum), the remaining peptides mixed with neutralized sinapinic acid are digested directly on the probe tip by 1 μ1 of carboxypeptidase P (Boehringer Mannheim Corp, Indianapolis, IN) and incubated at 23°C for 30 min. The sample is analyzed by mass spectrometry. Figure 3 shows a composite mass spectra of the peptide before (lower profile) and after (upper profile) *in situ* digestion by carboxypeptidase P in the presence of neutralized energy absorbing molecules. The decrease in mass represents the removal of a Gly residue from the C-terminal of the peptide.--

Please replace the paragraph beginning at page 50, line 15, with the following:

--Polypropylene or polystyrene or polyethylene or polycarbonate are melted in an open flame and deposited as a thin layer on a 2 mm diameter stainless steel probe element so as to cover it completely. Solid glass rod or solid nylon filaments (up to 1.5 mm diameter) or polyacrylamide rod are cut into 1 cm segments and inserted into the stainless steel probe support. Magnetic stir bars (1.5 x 8 mm, teflon-coated) are inserted into stainless steel probe tip support.

Appl. No. 10/700,297 Amdt. dated August 1, 2007

Response to Notice of Allowance of May 2, 2007

An aliquot of 1 µl of peptide mixture containing (GHHPH)₅G (SEQ ID NO:2) and human estrogen receptor dimerization domain, is mixed with 2 µl of dihydroxybenzoic acid (dissolved in 30% methanol, 0.1% trifluoroacetic acid) on each of such probe elements and analyzed by laser desorption time-of-flight mass spectrometry. Figure 4 shows that analytes could be desorbed from several examples of insulating, biocompatible surfaces.--

Please replace the paragraph beginning at page 76, line 22, with the following:

--2. Sequential *in situ* reactions are readily accomplished on samples deposited on top of an EAM surface. Thiosalicylic acid co-ordinate covalently bound to IDA-Cu(II) on a probe surface is prepared as described in Section 2.1. An aliquot of 1 μl of (GHHPH)₅G (SEQ ID NO:2) peptide is deposited on the surface and analyzed by laser desorption time-of-flight mass spectrometry. After obtaining several spectra (each an average of 50 laser shots), the sample is removed. An aliquot of 2 μl of carboxypeptidase Y (Boehringer Mannheim) is added directly on the surface and incubated at 37°C in a moist chamber for 5 min to 1 hr. The *in situ* enzyme digestion is terminated by 1 μl of 0.1% trifluoroacetic acid and the sample is reanalyzed by mass spectrometry.--

Please replace the paragraph beginning at page 82, line 2, with the following:

--A demonstration of this principle is provided by the actual amino acid sequence determination of a 26-residue peptide:

This peptide (GHHPH)₅G (SEQ ID NO:2) defines the metal-binding domain within the intact sequence of the 80-kDa protein known as histidine-rich glycoprotein (HRG).--

Appl. No. 10/700,297 Amdt. dated August 1, 2007 Response to Notice of Allowance of May 2, 2007

Please replace the paragraph beginning at page 82, line 8, with the following:

_--Glass beads with surface arylamine groups as coupling ligands (Sigma) are washed with and suspended in cold 0.3M HCl. A 50 mg/mL aqueous solution of NaNO2 is added to the beads at a ratio of 1:5 (v/v) (NaNO₂:HCl) and incubated at 4°C for 15 minutes with gentle shaking. After incubation, the beads are washed with cold 0.3M HCl and 50 mM sodium phosphate buffer pH 8.0. The peptide to be sequenced is added to the beads in sodium phosphate buffer at pH 8.0 and incubated for 24 hrs. at 40°C with gentle shaking. The beads with coupled peptides are washed with sodium phosphate buffer, sodium phosphate buffer with high concentration of salt (e.g., 1.0 M), dilute acid and organic solvent (e.g., methanol) until no peptide signal is detected in the supernate by MALDI-TOF mass spectrometry (one skilled in the art knows SEND, SEAC, and SEPAR may also be used) or by absorbance at 220 nm. An aliquot of 1 µL of the beads is then deposited on the probe tip, 1 µL of sinapinic acid (dissolved in 50% methanol/0.1% trifluoroacetic acid) is mixed with the beads and the sample was analyzed by laser desorption time-of-flight mass spectrometry. After obtaining several spectra (each an average of 50 laser shots), the remaining peptides on the surface are washed free of sinapinic acid with methanol and then digested with carboxypeptidase Y (Boehringer Mannheim) at 23°C in a moist chamber. The digested peptides are next washed with phosphate buffered saline (PBS) pH 8.0. An aliquot of 1 µL of sinapinic acid is added to the surface and analyzed again by laser desorption time-offlight mass spectrometry. The result of the C-terminal sequence analysis of the GHHPHG (SEQ ID NO:4) sequence is shown in Figure 35. A nascent sequence of the peptide is observed. The sequence is deduced by the differences in the mass between two peaks.--

Please insert the accompanying paper copy of the Sequence Listing, page numbers 1-2, at the end of the application.